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Short communication

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Modelling of transparency of Lake Baikal inferred from the Sentinel-2 Data

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ABSTRACT. The remote sensing methods usage makes it possible to increase the accuracy and efficiency of data on the state of water bodies. Among the many satellite systems, Sentinel-2 is the most suitable for inland water assessment. One of the abiotic factors in assessing the trophicity of water bodies is the transparency along the Secchi disk. Models for calculating water transparency have been developed for individual water bodies. The analysis showed that these models don't adequately describe the transparency for Lake Baikal. Based on the correlation-regression analysis, the parameters of the exponential function were estimated for calculating the transparency of the surface waters of Lake Baikal using the values of the Sentinel-2 spectral channels. Despite the inaccuracy of the model for assessing the transparency in the coastal zone, it can be used to assess the seasonal and interannual transparency of the surface waters of Lake Baikal.

Keywords: remote sensing, Secchi disk, water transparency, model, Sentinel-2, Baikal

1. Introduction

Field studies of reservoirs in situ are highly accurate, but they are carried out fragmentarily, most often irregularly and with low efficiency, and require high costs associated with the involvement of highly qualified specialists. The introduction of automatic ground stations for collecting information about water bodies to some extent solves these problems, but global coverage remains unavailable.

Over the past several decades, remote sensing and geographic information technology (GIS) have been successfully used in research to monitor surface water quality. This is reflected in the publications of A.A. Dontsov et al. (2017a; 2017b; 2018), S.J. Gray (1992), K. Ismail et al. (2019), and T.I. Kutyavina et al. (2019; 2020). Sensors installed on satellites and aircrafts measure the amount of radiation at different wavelengths reflected from the surface of water bodies, which is then converted into various indicators of water quality. Thanks to advances in remote sensing, parameters such as the concentration of suspended matter (TSS), chlorophyll *a*, turbidity and Secchi disk transparency (SDD) are well evaluated.

For the analysis, the data of various satellite remote sensing systems were considered on the parameters of openness, accessibility, spatial resolution, efficiency and quality of spectral channels. Today, the main competing systems for these criteria are Landsat and Sentinel (Fig. 1). Sentinel-2 is distinguished by an optimal balance of efficiency, accuracy and availability.

2. Materials and methods

One of the abiotic indicators of the trophicity of water bodies is the water transparency indicator using the Secchi disk. At the moment, various models have been developed to calculate the transparency of water for certain bodies of water.

For example, according to J. Delegido et al. (2019), the Secchi Disc Transparency Index (SD) is calculated as follows:

$$SD = 4.7134 * (R490/R560)^{2.5569} \quad (1)$$

where R490, R560 are MSI / Sentinel-2 spectral channels with the corresponding wavelength in nm.

Another approach to calculating transparency is described by M. Bonansea et al. (2019):

$$SDT = 1.79 - 134.15 * Band_{RE1} + 157.72 * Band_{NIR} + 0.53 * \left(\frac{Band_{RE3}}{Band_{NIRn}} \right) \quad (2)$$

where BandRE1, BandNIR, BandRE3, BandNIR are MSI / Sentinel-2 spectral channels that have undergone atmospheric correction.

The data of the expeditions of the Limnological Institute SB RAS was used to check the data of the transparency models for the Secchi disk. Data collection was carried out during spring and autumn expeditions. The locations of the stations for determining the transparency by the Secchi disk (SD, m) are shown in Figure 2.

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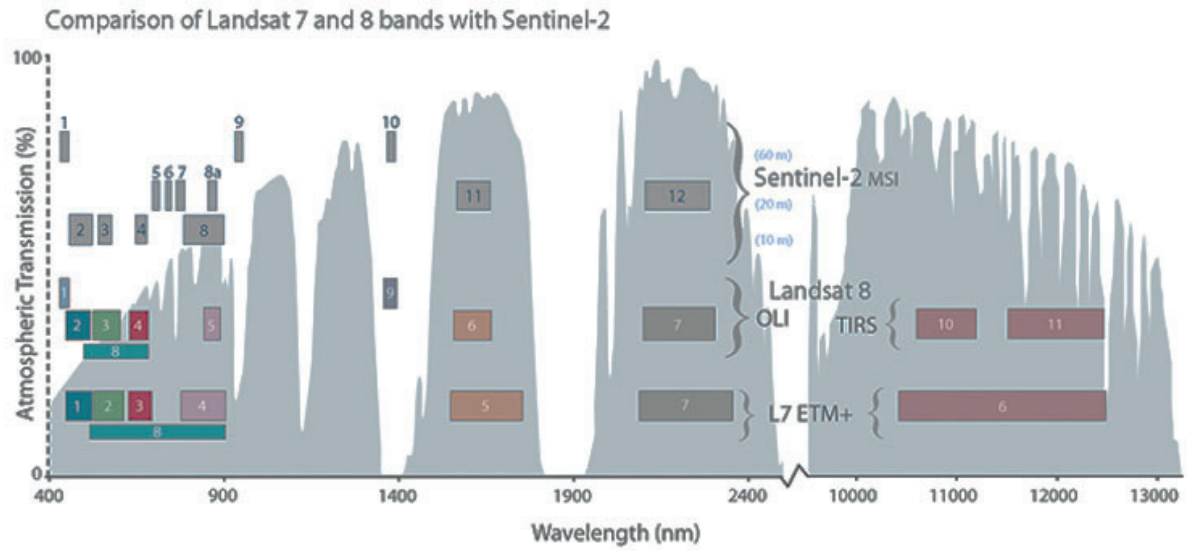

Fig.1. Comparison of Landsat 7 and 8 bands with Sentinel-2 (Image credit: U.S. Geological Survey).

Next, we selected Sentinel-2 satellite images that were close in terms of the date of measurements. Spectral channel data were extracted for further analysis. Since the researchers used linear and power dependences as models for assessing the transparency of reservoirs along the Secchi disk, the correlation dependences between the influencing factors and the studied indicator were checked.

We adapt the model suggested by J. Delegido et al. (2019) under our data. The coefficients of the model were obtained as a result of regression analysis:

$$SD = 6.7327\ (B02/B03)^{0.6875} \quad (3)$$

where B02, B03 are the corresponding Sentinel-2 spectral channels.

The coefficient of determination R2 = 0.10, which is considered a very low indicator, explaining that the studied indicator is described by influencing factors only by 10%. Fisher's criterion indicates the reliability of the model (6.99 > 3.99), the coefficients of the model are statistically significant according to the Student's test (21.00 > 2.00; 2.64 > 2.00). The average relative error of approximation was 42%. The results are far from those indicators of the quality of the model, which were indicated by the authors of the model.

Let's check the model of M. Bonansea et al. (2019). The coefficients of the model were obtained as a result of regression analysis:

$$SDT = 13.87 - 835.39 * B05 + 455.87 * B08 + 1.13 * \left(\frac{B07}{B8A}\right) \quad (4)$$

where B05, B08, B07, B8A are the corresponding Sentinel-2 spectral channels.

The coefficient of determination is higher (R2 = 0.40), Fisher's test indicates the unreliability of the model (13.97 > 3.99), the last coefficient of the model is statistically insignificant according to Student's test (0.35 < 2.00). The average relative error of approximation was 33%. The influencing factors are very closely interconnected, which violates the requirements for building a linear model.

Difficulties with the use of existing models lead to the need to develop your own model.

3. Results and discussion

An assumption was made based on the analysis of correlation dependences that the exponential function using the values of the spectral channels B05 and B07 is better suited to describe these dependences.

The dependences are the closest between ln (SD) and the values of these spectral channels (Fig. 3).

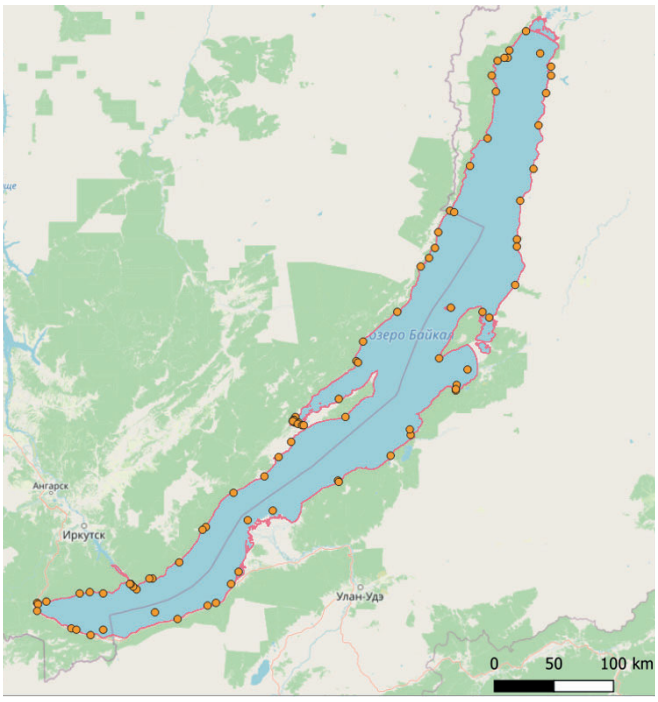

Fig.2. Locations of stations for determining the transparency by the Secchi disk (SD, m).

The task was to estimate the parameters of an exponential function of the form:

$$SD = a_0 * e^{(a_1 * B05 + a_2 * B07)} \quad (5)$$

As a result of the regression analysis, the following model was obtained:

$$SD = 22,8158 * e^{(-142,6480 * B05 + 75,0105 * B07)} \quad (6)$$

Determination coefficient R2 = 0.55. Fisher's criterion indicates the reliability of the model (38.98 > 3.99), the coefficients of the model are statistically significant according to the Student's test (23.00 > 2.00; 5.05 > 2.00; 2.89 > 2.00). The average relative error of approximation was 29%. As we can see, this model shows the best results.

This model can be used to calculate transparency for images of other periods (Fig. 4, Fig. 5).

In the future, it is possible to increase the accuracy of the model with an increase in the number of field observations.

There are disadvantages: there can be a very large error in the coastal zone. According to experts, this is influenced by a strong reflection from the bottom. In addition, the depth of the reservoir must be taken into account. This method cannot be applied to shallow water bodies.

4. Conclusions

It becomes possible to build transparency maps for the Secchi disk based on the obtained model on Lake Baikal during the absence of expeditions, to analyze the dynamics of this transparency change. Analysis of seasonal and interannual changes will make it possible to draw conclusions about the cyclicality, the influence of the anthropogenic factor and climatic changes.

Acknowledgements

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Conflicts of interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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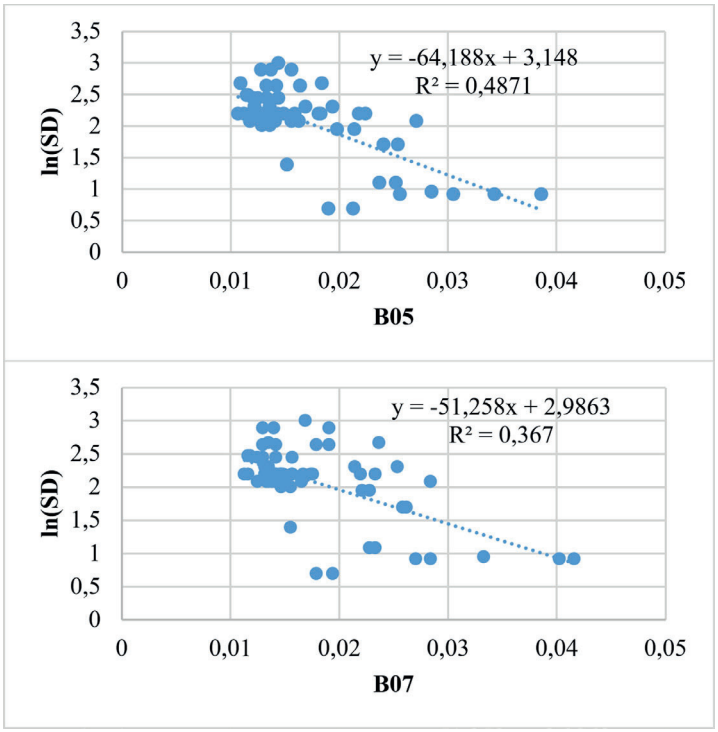

Fig.3. Relationship between ln (SD) and B05 and B07.

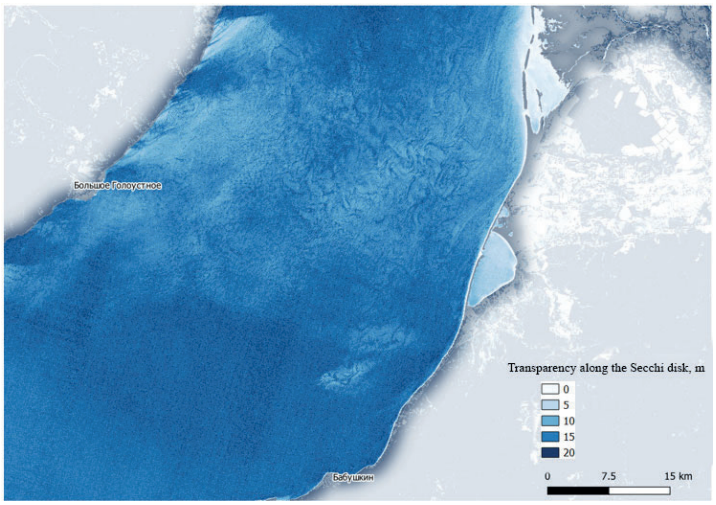

Fig.4. Transparency map using the Sentinel-2 satellite image on May 25, 2020

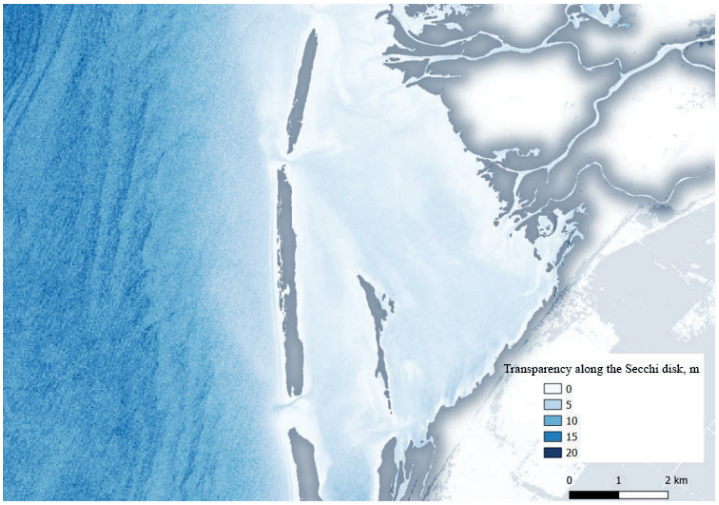

Fig.5. Fragment of transparency map using the Sentinel-2 satellite image on May 25, 2020.

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Original Article

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Variability and problem of species identification of sculpins of the genus *Cyphocottus* (Pisces: Cottidae)

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ABSTRACT. Based on the material collected during the fieldwork seasons from 1996 to 2007 in the amount of 223 specimens, we studied phenetic relationships and taxonomic structure of the genus *Cyphocottus*. Two valid species, *C. megalops* and *C. eurystomus*, within the taxonomic boundaries established by D.N. Taliev (1955) were confirmed in the genus *Cyphocottus*. Interspecific differences in *C. eurystomus* are manifested in greater values of interorbital distance, the height of the head, body and caudal peduncle, as well as in smaller values of eye diameter and caudal peduncle length. Polymorphism in the definitive sizes, colour and the number of neuromasts in the lateral line is typical of *C. eurystomus*. At the same time, all populations have specific characters of *C. eurystomus* different from *C. megalops*. We found no intraspecific variability in *C. megalops*.

Keywords: Baikal endemic sculpins, genus *Cyphocott*us, phenetic and taxonomic relationships, Lake Baikal

1. Introduction

V.G. Sideleva founded the genus *Cyphocottus*, humpback sculpins, including two species, *C. megalops* and *C. eurystomus* (Sideleva, 2003). The name of the genus *Cyphocottus* originates from the Latinized Greek words "*cyphos*", which means humpback, and "*cottus*", which is sculpin, thus, reflecting the morphological feature of these fish. Genetic studies confirmed the validity of this genus (Kontula et al., 2003). Type species, *C. megalops*, was originally described within the genus *Cottus* (Gratzianov, 1902). Later, it was placed within the genera *Limnocottus* (Berg, 1906; Sideleva, 1982), *Abyssocottus* (Gratzianov, 1907) and *Asprocottus* (Taliev, 1955). Overall, during the studies, four species were described: *Cottus megalops* (Gratzianov, 1902); *Limnocottus megalops elegans* (Taliev, 1948) and *Asprocottus megalops eurystomus*, which included two subspecies, namely the "type" subspecies inhabiting the Selenga shallow water, Barguzin Bay and the Maloye More Strait and the "Southern Baikal" subspecies inhabiting, as follows from its name, the southern basin of Lake Baikal. The latter subspecies differs from the former one by a variegated colour and some plastic and meristic characters (Taliev, 1955). Subsequently, the name *C. eurystomus* was entrenched not only for this subspecies but also for all spotty individuals as well as the name *C. megalops* – for monochromic individuals (Sideleva, 2003).

The confusion in the identification of taxa is due to two circumstances. First, the original description of *C. megalops* was made for an juvenile individual with exterior features that were not completely formed. Second, for a long time, there were no new findings of this species. Between the first finding in 1891, "the second discovery" of the species in 1943 and our samplings from 2000 to 2007, the intervals were more than half a century. The collected material allows us to solve the problems concerning the identification of taxa and consider their intraspecific variability.

2. Material and methods

This study was based on the material collected from 1996 to 2007. A total of 223 specimens were examined (Table 1). The species were identified by characters indicated by D.N. Taliev (1955) and V.G. Sideleva

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Table 1. List of samples and analyses

No. of samples	Name of locality	Date	Number of neuromasts	Other morphological characters
	Cyphocottus megalops			
1	Chivyrkuy Bay estuary	16 August 2006	1	1
2	Barguzin Bay	19 June 2000	4	5
3	the Selenga shallow water	27 May 2007	7	7
	the Selenga shallow water	17 June 2000	-	9
4	area near the estuary of the Buguldeika River	08 June 2003	10	18
	Cyphocottus eurystomus (monochromic form)			
5	area near the estuary of the Kichera River	02 June 2007	5	5
6	Frolikha Bay	04 June 2002	-	9
7	Tompa Bay	05 June 2002	-	14
8	Shegnanda Cape	06 June 2002	5	5
9	Bolshiye Vorota Strait	November 2004	25	25
	Bolshiye Vorota Strait	30 May 2007	6	6
10	Khoboy Cape	19 June 2000	3	8
11	the Selenga shallow water	29 October 2004	30	30
	Cyphocottus eurystomus (spotty form)			
12	Chivyrkuy Bay estuary	20 September 1999	11	11
13	area near the estuary of the Buguldeika River	08 June 2003	25	25
14	Gremyachinsk Cape	March 2005	10	10
15	Bolshaya Kosa Cape	07 June 2002	1	1
	Solontsovaya Bay	22 June 2000	1	1
16	Ushkany Islands	21 June 2000	1	6
	Ushkany Islands	07 June 2003	1	1
17	Bolshiye Koty Bay	July 2003	1	1
	Listvenichny Bay	13 February 1996	5	5
	Listvenichny Bay	December 2000	3	6
	Listvenichny Bay	December 2001	3	5
	Listvenichny Bay	May 2002	7	7
	area near the Slyudyanka town	March 2000	2	2

(2003). Due to discrepancies in the identification of *C. megalops* in the above references, the two forms were compared for compliance with the characters of the holotype descripted by V.I. Gratzianov (1902) for ten plastic characters. As an alternative, the following was used: the *C. megalops* (sensu Sideleva, 2003) selection consisting of 15 juvenile individuals collected in the northern part of Lake Baikal, Frolikha and Tompa bays (form A in Table 2) and the *C. megalops* (sensu Taliev, 1955) selection consisting of 40 adult individuals from different areas of Lake Baikal (form B in Table 2). The study of interspecific and intraspecific variability was carried out on 17 samples by 17 meristic and 29 plastic characters. The selections were compared by principal component analysis (PCA) technique using the SPSS 8.0 software (Laerd Statistics, 2015)

3. Results and discussion

Identification of taxa

The analysis of phenetic relationships of the juvenile *C. megalops* (sensu Sideleva, 2003) specimens, the adult *C. megalops* (sensu Taliev, 1955) specimens and the *C. megalops* holotype has revealed that these selections diverge in the space of principal components by such characters as eye diameter, the height of body and head in the space of the first principal component as well as by the postorbital distance and the interorbital distance, head length and caudal peduncle length in the space of the second principal component (Table 2). The *C. megalops* holotype occupied the centre on the scatter plot of the *C. megalops* (sensu Taliev, 1955) selection (Fig. 1). Therefore, the identification of taxa proposed

by D.N. Taliev (1955) should be considered correct, to which we will adhere below.

The distinctive features of *C. megalops* are an oblong body, large oval eyes (eye diameter is usually larger than the snout length) and monochromic colour: the brown back, yellow-brown sides and yellowish-grey fins (Fig. 2A). The distinctive features of *C. eurystomus* are a tall fusiform body forming a hump behind the occiput and round eyes with a diameter smaller than the snout length. The colour of the body and fins varies significantly (Fig. 2B-2E); therefore, the division into monochromic and spotty forms is rather nominal.

Variability of definitive sizes

There were no differences in the sizes of adult *C. megalops* individuals (Table 3). The total length (TL) of the studied specimens ranged from 93 to 127 mm. D.N. Taliev (1955) gave similar data (110-120 mm). In contrast, the sizes of *C. eurystomus* are subject to significant variability. Four size groups can be distinguished: large (TL up to 215, 160-185 mm on average) individuals inhabiting the Selenga shallow

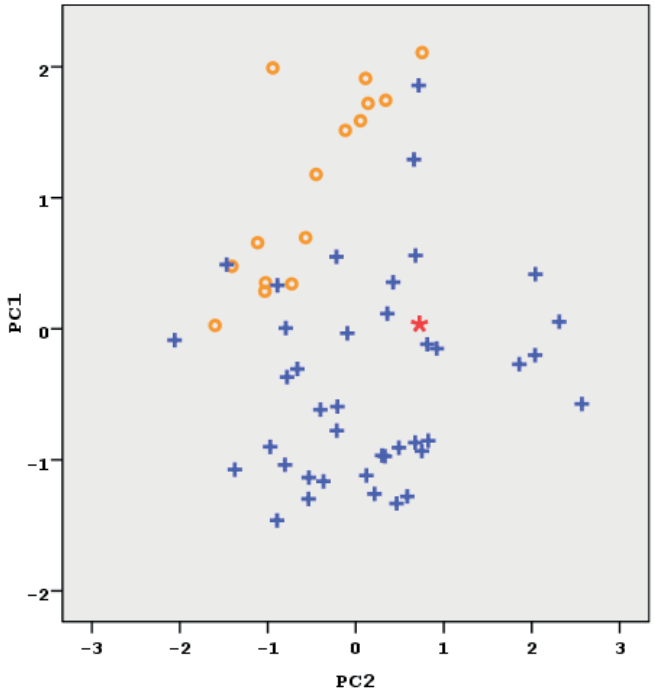

Fig.1. Phenetic relationships between the holotype of C. *megalops* and potentially conspecific specimens: red star – holotype of C. *megalops,* orange circles – juvenile specimens of C. *megalops* sensu Sideleva (2003), blue cross – adult specimens of C. *megalops* sensu Taliev (1955).

Table 2. Values of the key morphological characters and component score coefficient matrix for analysis of the *Cyphocottus megalops* identification

	Holotype of *C. megalops*	Examined forms		Principal Component	
		A	B	1	2
Total length (mm)	71.0	74.2 63.6-84.2	110.9 93.2-127.1	% of Variance	
Standard length (mm)	60.0	60.8 52.4-69.3	92.4 76.0-107.1	38.340	16.777
Plastic characters in % of standard length					
head length	31.7	34.3 ± 0.77 33.0-35.8	31.7 ± 0.91 29.6-33.9	0.188	-0.218
height of the trunk	18.3	20.0 ± 1.43 17.5-21.9	15.7 ± 2.28 12.2-21.7	0.232	-0.136
height of caudal peduncle	5.0	5.5 ± 0.29 5.1-6.0	5.4 ± 0.35 4.4-6.3	0.156	0.231
length of caudal peduncle	19.2	16.9 ± 1.07 15.2-19.2	16.4 ± 1.21 13.7-18.8	-0.003	-0.373
Plastic characters in % of head length					
snout length	28.9	27.9 ± 1.36 25.5-31.3	26.9 ± 1.78 19.7-30.9	0.094	0.155
longitudinal eye diameter	31.6	24.7 ± 1.44 21.9-27.1	27.3 ± 2.12 21.7-32.4	-0.176	0.017
postorbital distance	44.7	41.1 ± 1.66 37.9-44.5	41.6 ± 1.74 37.5-46.1	0.041	0.438
width of the head	63.2	70.4 ± 4.68 63.2-79.3	63.8 ± 9.73 53.0-94.7	0.166	-0.166
head height near occiput	50.0	52.8 ± 2.84 47.2-56.0	46.7 ± 4.18 41.0-61.1	0.235	-0.050
interorbital distance	10.5	7.2 ± 1.50 5.2-9.6	6.2 ± 1.54 3.4-11.0	0.151	0.302

Note: Above the line – average values and standard deviations, below the line – limits of variability of the characteristic value. Symbols: form A – juveniles of *C. megalops* sensu Sideleva, 2003; form B – *C. megalops* sensu Taliev, 1955.

water and the northern part of the Maloye More Strait; medium individuals (TL up to 165 mm, 130-145 mm on average); small individuals (TL up to 135-145 mm, 100-125 mm on average) and dwarfs (TL up to 96 mm). Small and medium individuals are found throughout Lake Baikal; dwarfs were found only at one site, near Khoboy Cape at a depth of 100 m.

Variability of morphometric characters

The study of the variability in the number of neuromasts in sensory lines has revealed the absence of interspecific differences. At the intraspecific level, in *C. megalops* and monochromic form of *C. eurystomus*, the number of neuromasts in the lateral line increases from the northern to the southern part of the range (Table 3). The greatest number of neuromasts is typical of the *C. eurystomus* population in the Selenga shallow water. The scatter plot of the selections in the space of principal components (Table 4, Fig. 3A) shows this as a displacement of the scatter area of this population along the axis of the second principal component to the right side of the plot.

Analysis (PCA) of other morphometric characters showed that body height, caudal peduncle height, head height near occiput, and interorbital distance yield the greatest positive loading on the first principal component, and caudal peduncle length and eye diameter – the negative one. These characters determine interspecific differences. Body length yields the greatest positive loading on the second principal component, and head length – the negative one. These characters determine the difference of *C. eurystomus* in the Selenga shallow water from other populations of this species (Table 5, Fig. 3B).

4. Conclusions

This study has revealed interspecific differences between *C. megalops* and *C. eurystomus* manifested in greater values of interorbital distance, the height of the head, body and caudal peduncle, as well as smaller values of eye diameter and caudal peduncle length, in *C. eurystomus*. The population of *C. eurystomus* in the Selenga shallow water differs from other populations of this species by the same characters. Therefore, this population and *C. megalops* represent the

Table 3. Component score coefficient matrix for analysis of the number of neuromasts in the sensory lines of 17 samples of the *Cyphocottus* sculpins

Characters	Principal Component	
	1	2
	% of Variance	
	37.181	15.540
number of neuromasts in a left supraorbital line	0.140	-0.151
right supraorbital line	0.150	-0.176
left infraorbital line	0.155	-0.012
right infraorbital line	0.151	-0.049
left temporal line	0.131	-0.052
right temporal line	0.140	-0.106
left occipital line	0.118	0.063
right occipital line	0.137	0.063
left preopercular-mandibular line	0.157	-0.107
right preopercular-mandibular line	0.159	-0.023
left lateral line	0.090	0.470
right lateral line	0.085	0.474

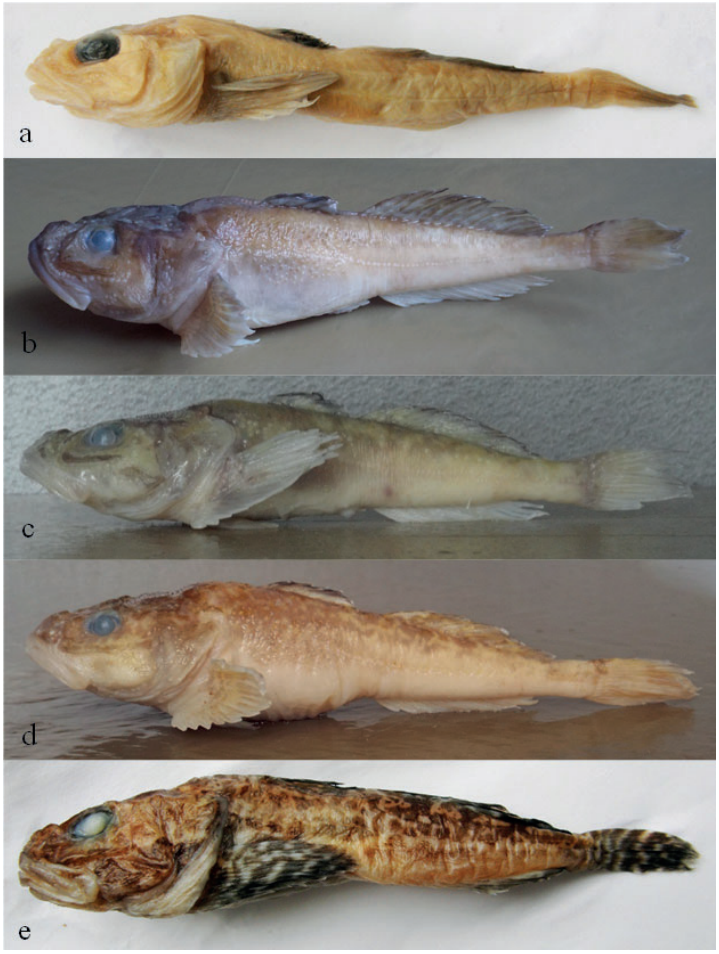

Fig.2. Variability of colours: A. C. *megalops;* B-E. C. *eurystomus:* (B and C) – monochromic form; (D and E) – spotty form.

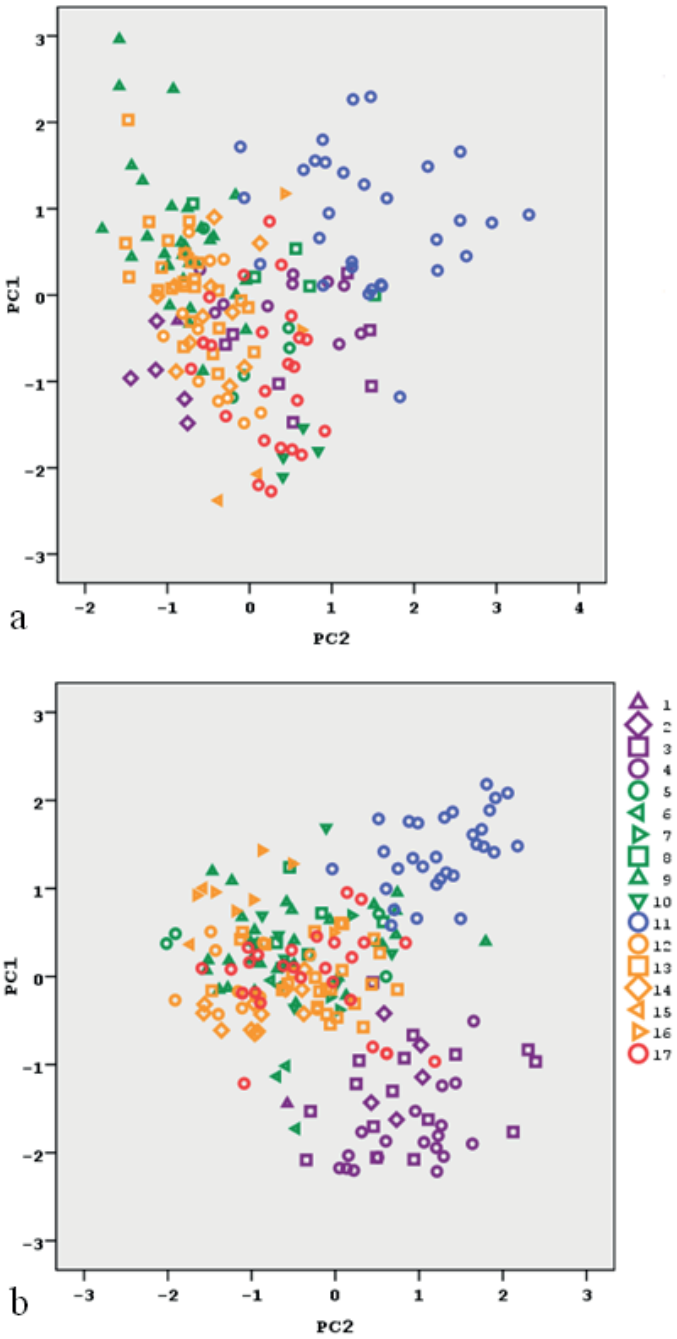

Fig.3. Distribution of 17 samples of sculpins of the genus *Cyphocottus* in the space of the first (PC1) and second (PC2) principal components: A. by numbers of neuromasts; B. by other meristic and plastic characters. Numeration of samples as in Table 1. Colour of symbols: violet – *C. megalops*; green and blue – a monochromic form of *C. eurystomus* (blue – the Selenga shallow water as the type locality); orange and red – a spotty form of *C. eurystomus* (red – the southern basin of Lake Baikal as the type locality).

Table 4. Component score coefficient matrix for analysis of morphological characters of 17 samples of the *Cyphocottus* sculpins

Characters	Principal Component	
	1	2
	% of variance	
	28.480	9.962
Meristic characters		
number of rays in the first dorsal fin	-0.011	0.070
second dorsal fin	0.012	0.169
left pectoral fin	0.056	-0.085
anal fin	0.026	0.130
number of gill rakers	-0.027	0.009
Plastic characters in % of standard length		
head length	0.032	-0.230
length of the trunk	0.015	0.192
height of the trunk	0.088	-0.053
height of caudal peduncle	0.080	0.045
width of the trunk	0.071	-0.047
antedorsal distance	0.046	-0.196
postdorsal distance	-0.034	0.092
anteventral distance	0.030	-0.130
anteanal distance	0.007	-0.167
length of the caudal peduncle	-0.068	0.002
pectroventral distance	0.063	-0.008
ventroanal distance	-0.001	-0.036
length of insertions of the first dorsal fin	0.019	-0.007
second dorsal fin	0.043	0.034
height of the first dorsal fin	-0.002	-0.058
second dorsal fin	0.056	0.056
length of insertions of the anal fin	0.056	0.093
height of anal fin	0.048	0.093
length of pectoral fin	0.062	0.089
length of ventral fin	0.020	0.053
Plastic characters in % of head length		
snout length	0.067	0.026
longitudinal eye diameter	-0.079	0.050
postorbital distance	0.071	0.051
width of the head	0.075	-0.043
head height near occiput	0.087	0.000
head height near eye	0.074	0.043
interorbital distance	0.088	0.006
length of upper jaw	0.076	0.041
length of lower jaw	0.048	0.032

Table 5. Size of specimens and values of the key morphometric characters of the *Cyphocottus* sculpins samples

№ of samples	Characters										
	TL	SL	*l.l.*	*c*	*H*	*h*	*pl*	*ao*	*o*	*hcz*	*io*
1	98.2	81.5	51-58	33.7	20.1	5.3	18.8	27.3	27.3	52.7	6.5
2	117.6 98.9-127.1	98.9 83.9-105.2	39-59	32.0 ± 0.81 30.7-33.0	16.2 ± 0.99 14.7-17.2	5.4 ± 0.35 4.7-5.7	16.2 ± 1.16 14.7-18.0	27.7 ± 1.82 25.6-30.9	24.4 ± 2.10 21.7-26.3	49.2 ± 3.55 44.4-53.8	7.4 ± 1.30 5.3-8.5
3	108.1 93.2-125.0	89.2 76.0-103.0	73.7 ± 11.5 55-89	31.9 ± 1.03 29.6-33.9	17.0 ± 2.33 13.7-21.7	5.4 ± 0.40 4.4-6.3	16.4 ± 1.34 13.7-17.9	26.7 ± 2.34 19.7-30.8	27.2 ± 2.17 23.8-32.4	48.6 ± 4.35 42.9-61.1	6.1 ± 1.73 3.4-11.0
4	112.1 98.6-125.6	94.0 82.8-107.1	75.4 ± 8.19 58-87	31.4 ± 0.58 30.2-32.5	14.0 ± 0.92 12.2-16.0	5.3 ± 0.31 4.9-6.1	16.4 ± 0.99 14.4-18.8	26.9 ± 0.99 24.9-28.8	28.2 ± 1.16 26.3-30.7	44.0 ± 2.01 41.0-49.7	5.9 ± 1.28 4.3-9.4
5	145.4 131.7-157.0	121.3 110.0-130.6	60-72	33.9 ± 1.19 32.0-35.5	20.9 ± 2.13 16.8-23.1	6.3 ± 0.26 5.8-6.5	15.5 ± 0.87 14.3-16.6	28.7 ± 1.02 27.6-30.5	19.8 ± 1.10 18.6-21.4	57.0 ± 2.98 51.7-60.4	12.9 ± 1.21 11.3-14.8
6	98.4 70.7-134.8	81.7 57.8-114.0	60-64	33.7 ± 0.44 33.1-34.4	18.5 ± 0.94 17.2-20.4	5.4 ± 0.23 5.0-5.7	17.2 ± 1.40 14.7-19.2	27.1 ± 1.16 25.1-28.6	24.0 ± 2.45 18.4-26.4	50.9 ± 2.91 47.2-56.1	7.1 ± 2.03 5.2-11.6
7	85.0 63.6-155.7	70.3 52.4-132.9	56-68	34.0 ± 1.10 32.0-35.8	20.5 ± 0.85 19.0-21.9	5.5 ± 0.37 4.9-6.1	16.2 ± 0.92 14.1-18.0	28.2 ± 1.55 26.3-31.3	23.6 ± 2.07 19.5-27.1	54.8 ± 2.02 50.5-59.2	8.5 ± 1.92 5.6-12.6
8	133.5 124.0-151.0	111.0 103.4-121.7	63-86	33.5 ± 0.92 32.4-34.4	22.4 ± 1.47 20.1-24.2	6.1 ± 0.23 5.8-6.4	15.7 ± 0.87 14.7-17.3	29.3 ± 1.46 27.5-31.9	21.9 ± 1.44 20.3-24.4	60.5 ± 2.40 57.0-64.1	11.6 ± 1.10 10.4-13.6
9	160.1 135.0-181.5	135.1 112.2-153.5	61.5 ± 4.22 52-72	34.1 ± 1.15 32.0-36.8	20.6 ± 1.36 18.3-23.9	6.4 ± 0.50 5.5-7.7	13.8 ± 1.08 11.5-15.9	28.4 ± 0.96 26.1-30.2	19.6 ± 1.11 16.9-21.7	53.7 ± 3.13 46.0-60.2	11.9 ± 1.49 9.4-15.2
10	88.9 76.0-96.1	74.9 64.5-81.8	64.8 ± 4.21 60-71	34.4 ± 0.80 33.2-35.6	23.7 ± 1.61 20.5-25.6	5.7 ± 0.86 4.9-7.8	13.6 ± 1.37 11.3-15.4	27.6 ± 0.83 26.5-28.9	22.5 ± 1.60 19.5-24.8	54.2 ± 3.45 49.4-60.9	11.2 ± 1.60 9.6-14.3
11	185.8 154.7-214.5	156.2 129.5-181.0	91.7 ± 11.7 71-122	32.5 ± 0.84 30.8-34.3	23.0 ± 1.33 20.7-25.7	7.0 ± 0.46 5.7-7.9	13.6 ± 0.87 12.1-15.1	30.5 ± 1.18 28.1-33.6	19.4 ± 1.58 15.2-22.1	60.1 ± 3.00 52.5-67.2	14.6 ± 1.83 10.8-18.4
12	124.6 113.3-141.2	104.1 94.6-119.0	60.0 ± 3.69 54-67	34.6 ± 0.98 32.0-35.8	19.9 ± 0.88 18.6-21.3	6.1 ± 0.48 5.5-7.2	15.1 ± 1.10 13.4-16.8	28.5 ± 1.00 26.5-30.4	22.1 ± 1.67 20.1-26.2	53.3 ± 3.50 49.1-61.4	10.4 ± 1.61 7.9-13.4
13	144.8 110.8-165.6	123.3 93.9-143.0	58.3 ± 3.74 48-65	33.9 ± 1.09 31.5-36.1	20.5 ± 1.70 16.9-23.2	6.2 ± 0.39 5.5-7.0	14.7 ± 1.10 13.0-17.6	28.3 ± 1.03 26.3-30.3	21.9 ± 1.26 18.6-24.5	53.3 ± 2.89 46.9-60.0	11.5 ± 0.91 10.0-13.9
14	124.6 113.7-136.0	107.3 96.6-119.1	60.9 ± 5.16 51-71	33.9 ± 0.69 33.0-35.4	19.1 ± 0.89 17.4-20.6	5.6 ± 0.30 5.2-6.4	14.4 ± 1.02 12.8-16.0	27.9 ± 0.81 26.3-29.7	24.1 ± 1.55 20.8-26.8	53.5 ± 2.25 50.0-57.0	10.5 ± 1.10 8.9-12.3
15	74.4-91.1	61.5-77.0	50-54	34.3-37.0	21.6-25.2	5.2-5.8	13.5-16.3	27.9-33.7	20.3-24.6	53.7-56.6	9.5-14.7
16	122.5 117.7-131.3	103.3 98.1-111.3	73-74	34.6 ± 0.84 33.5-35.7	25.0 ± 1.86 22.1-27.5	5.6 ± 0.31 5.0-5.9	14.3 ± 0.62 13.4-15.1	30.4 ± 1.18 28.8-32.6	21.0 ± 1.39 19.3-22.9	57.3 ± 3.54 51.6-61.1	12.8 ± 1.08 10.9-14.2
17	119.2 96.3-144.7	100.4 81.9-122.4	65.6 ± 4.69 54-72	34.5 ± 1.08 32.1-36.8	19.8 ± 1.88 14.7-22.8	5.6 ± 0.45 4.5-6.3	14.8 ± 1.12 11.9-16.9	28.1 ± 1.51 23.8-31.1	22.5 ± 0.87 20.7-23.9	54.0 ± 3.53 47.2-60.9	12.7 ± 2.03 10.5-18.5

Note: Above the line – average values and standard deviations, below the line – limits of variability of the characteristic value. Symbols: TL – total length, SL – standard lenth, *l.l.* – number of neuromasts in the lateral sensory line; *c* – head length, *H* – height of the trunk, *h* – height of caudal peduncle, *lpc* – length of caudal peduncle, *ao* – snout length, *o* – longitudinal eye diameter, *cH* – head height near occiput, *io* – interorbital distance. Numeration of samples as in Table 1.

extreme forms of variability in sculpins of the genus *Cyphocottus*. The other selections, although filling the space between them, do not proceed to the intermediate form per se. They differ from each other by the number of neuromasts in sensory lines, colour and definitive sizes. Any combination of the selections, which is homogenous in one of these characters, will be heterogeneous in the rest. They all have the characters of *C. eurystomus* and differ from *C. megalops*. We have not found the intraspecific variability in *C. megalops*.

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Conflicts of interest

The author declare no conflicts of interest.

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PCR-screening of bacterial strains isolated from the microbiome of the *Lubomirskia baicalensis* sponge for the presence of secondary metabolite synthesis genes

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ABSTRACT. The microbial communities of sponges (Porifera) are often a source of natural bioactive metabolites. From the microbiome of the endemic *Lubomirskia baicalensis* sponge, 35 bacterial strains were isolated and identified using molecular methods. The strains belonged to the phyla Actinobacteria, Firmicutes, and Proteobacteria (classes Alpha- and Betapriteobacteria). To analyze the strains for the presence of genes in the synthesis of secondary metabolites, polyketide synthases (PKS), PCR screening was applied using degenerate primers. Overall, 15 out of 35 strains contained PCR products corresponding in size to a fragment of the ketosynthase domain of the PKS gene cluster. Thus, the proposed method is applicable for rapid screening of the potential ability of microorganisms of different taxonomic groups to produce secondary metabolites. The work contributes to the study of the taxonomic diversity of cultured microorganisms, potential producers of biologically active substances, isolated from the microbiomes of Baikal sponges.

Keywords: Lake Baikal, sponges, *Lubomirskia baicalensis*, bacterial strains, genes of bioactive metabolite synthesis, polyketide synthase, PCR-screening

1. Introduction

Symbiotic associations between sponges and microorganisms have existed for over 600 million years (Wilkinson et al., 1984; Love et al., 2009). Sponges are an important component of the benthic fauna of the world's oceans, as well as many freshwater habitats (Hooper and van Soest, 2002; Bell, 2007). These animals are effective filter feeders: the volume of water that a sponge pumps per day can 10,000 times exceed its body volume (Weisz et al., 2008). Sponge microorganisms average about 35% of the animal biomass, reaching 70% in some species (Taylor et al., 2007; Ribes et al., 2012). Many metabolites of bacterial origin (such as antibiotics, toxins or statins) are known to be polyketides and synthesized by multienzyme complexes, polyketide synthases (PKS). Such multi-enzyme complexes use acyl-coenzyme-A monomers as a substrate and consist of several proteins,"building blocks" (Ehrenreich et al., 2005; Barrios-Llerena et al., 2007). Each protein has a domain structure and, accordingly, several active centers. A group of domains responsible for one condensation cycle forms a "module" consisting of at least three domains: ketosynthase (KS), acyltransferase (AT) and acyl-carrying protein (ACP) (Jenke-Kodama and Dittmann, 2009). Since the sequences of modules in PKS systems correspond to gene clusters in the genomes of microorganisms, it is possible to detect the ability of communities of microorganisms and their individual strains to produce bioactive components using PCR detection of these genes. Notably, bacterial strains obtained from unusual and unexplored communities are often the source of new bioactive metabolites (Jenke-Kodama and Dittmann, 2009). The large species richness of sponges inhabiting Lake Baikal (18 species, 14 of which are endemic) is associated with a variety of ecological niches and habitat conditions (Kozhov, 1962). Therefore, an important scientific direction is a research aimed at identifying the ability of microorganisms of the Baikal sponges to produce bioactive metabolites. In this work, we identified 35 bacterial strains isolated from the endemic Baikal sponge, *Lubomirskia baicalensis*, and also performed PCR screening of these strains for the presence of fragments of the gene from the PKS ketosynthase domain in their genomes.

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2. Material and methods

Samples of *L. baicalensis* were collected in August 2011 in the area of the Listvyanka settlement (southwest coast of Lake Baikal) from a depth of 15 m using diving equipment. Bacterial strains from the sponge were isolated out on the R2A agar medium (Becton Dickinson, United States) according to the previously published method (Parfenova et al., 2008). DNA from bacterial cultures was isolated using the RiboSorb kit according to the manufacturer's instructions (AmpliSens, Russia). The strains were identified by analyzing the sequences of 16S rRNA genes amplified using the 9F and 1093R eubacterial primers, as described in our previous article (Kaluzhnaya et al., 2012). The primers for PCR screening of polyketide synthase (PKS) genes were selected based on literature data (Ehrenreich et al., 2005). Comparison with databases of nucleotide sequences was carried out using the BLASTX program of the NCBI server.

3. Results

Thirty-five bacterial strains isolated from the *L. baicalensis* freshwater sponge in August 2011 were identified by the 16S rRNA gene sequences. The collection contains representatives of 16 genera belonging to three bacterial phyla (Table): Firmicutes, Actinobacteria, and Proteobacteria (classes Alphaproteobacteria and Betaproteobacteria). The table shows the homology of the obtained 16S rRNA sequences with those published in the NCBI database (http://www.ncbi.nlm.nih.gov/). Most of the sequences demonstrated 97-100% nucleotide sequence identity with known bacterial strains.

Overall, 17 strains were assigned to the phylum **Actinobacteria** identified as *Janibacter* sp. 04-Lb11/2, *Promicrospora* sp. 05-Lb11/2, *Arthrobacter* sp. 06-Lb11/2, 07-Lb11/2, 13-Lb11/2, 20-Lb11/2, 32-Lb11/2, *Rathayibacter* sp. 08-Lb11/2, 11-Lb11/2, *Kocuria* sp. 12-Lb11/2, 31-Lb11/2, *Rhodococcus* sp. 14-Lb11/2, 33-Lb11/2, *Microbacterium* sp. 15-Lb11/2, 19-Lb11/2, 38-Lb11/2, and *Flexivirga* sp. 21-Lb11/2; 8 strains were assigned to the phylum **Proteobacteria**: *Methylobacterium* sp. 03-Lb11/2, *Massilia* sp. 17-Lb11/2, *Rhodopseudomonas* sp., 22-Lb11/2, 23-Lb11/2, 42-Lb11/2, *Heminiimonas* sp. 30-Lb11/2, and *Tardiphaga* sp. 36-Lb11/2 and 40-Lb11/2; and 10 strains - to the phylum **Firmicutes:** *Staphylococcus* sp. 10-Lb11/2, *Bacillus* sp. 16-Lb11/2, 18-Lb11/2, 24-Lb11/2, 35-Lb11/2, 37-Lb11/2, *Paenibacillus* sp. 26-Lb11/2, 27-Lb11/2, 28-Lb11/2, and 29-Lb11/2 (Table).

According to the results of PCR screening, a product corresponding in size (700 bp) to a fragment of the ketosynthase domain of the polyketide synthase gene was found in 15 out of 35 strains. Of these, 7 strains belonged to the phylum Actinobacteria; 5 strains - to the phylum Firmicutes, and 3 strains - to the phylum Proteobacteria (Table). These bacterial cultures are interesting for further research because they can produce secondary metabolites of a polyketide nature that are important for medicine and biotechnology.

The nucleotide sequences were deposited in GenBank under the accession numbers MZ646072-MZ646106.

4. Discussion

The members of the phylum **Actinobacteria** were the most numerous and diverse group among the isolated cultures. These microorganisms are widespread in both marine and freshwater communities and are one of the most studied groups of bacteria due to their importance in biotechnology, medicine, and ecology. They are efficient producers of new secondary metabolites that show a range of biological activities, including antibacterial, antifungal, anticancer, antitumor, cytotoxic, cytostatic, anti-inflammatory, anti-parasitic, anti-malaria, antiviral, antioxidant, anti-angiogenesis, etc. (Manivasagan et al., 2014; Lee et al., 2020). Actinobacteria were found in all previously studied communities of freshwater sponges, constituting a significant part of the bacterial 16S rRNA sequences in them (Kaluzhnaya et al., 2011; 2012; Kaluzhnaya and Itskovich, 2014; Gladkikh et al., 2014; Seo et al., 2016; Kulakova et al., 2018).

In our study, the PCR-signal was detected in the following actinobacterial strains: 04-Lb11/2 (*Janibacter* sp.), 08-Lb11/2 (*Rathayibacter* sp.), 11-Lb11/2 (*Rathayibacter* sp.) 14-Lb11/2 (*Rhodococcus* sp.), 15-Lb11/2, 38-Lb11/2 (*Microbacterium* sp.), and 20-Lb11/2 (*Arthrobacter* sp.). These bacterial cultures are interesting for further research because some related strains have shown the ability to produce secondary metabolites that are important for medicine and biotechnology. In the scientific literature, we can find many similar investigations.

For example, helquinoline that exhibits high antibacterial and antifungal activity was isolated by a group of German scientists from the *Janibacter limosus* strain Hel 1 ethyl acetate extract (Asolkar et al., 2004). Actinobacteria belonging to the genus *Rathayibacter* were capable of producing toxigenic glycoprotein, the tunicaminyluracil antibiotics (Tancos et al., 2019). Strains of the phytopathogenic *Rhodococcus fascians* bacteria are able to produce phytohormones, leading to the development of so-called leafy galls on a wide range of host plants (Nacoulma et al., 2013). It has been also shown that various *Rhodococcus* strains are involved in the synthesis of bioactive steroids (Haroune et al., 2004) as well as in the biodegradation of a wide range of organic components, including environmentally hazardous toxins, herbicides, naphthalene, toluene, biphenyl, etc. (Zhao et al., 2011).

Sponges are often a source of bacterial strains-producers of bioactive substances. For example, strains of the genus *Microbacterium* isolated from a marine sponge, *Halichondria panacea,* produced glucosylmannosyl-glycerolipid that inhibits the growth of tumor cells (Lang et al., 2004).

Analysis of 70 genomes belonging to 20 species of *Microbacterium* revealed that most of them contain gene clusters encoding pathways for the production of terpenoids, type III polyketide synthases and non-

Table. Bacterial strains isolated from sponge *L. baicalensis* (collection LB11/2), sampled in August 2011

Strain	Acc. No.	Closest homologues (Acc. No.)	Per. Ident, %	Phylum	PCR-sygnal (PKS)
03-Lb11/2	MZ646072	*Methylobacterium variabile* (AB900978)	100.0	Alphaproteobacteria	+
04-Lb11/2	MZ646073	*Janibacter limosus* (MN826598)	99.0	Actinobacteria	+
05-Lb11/2	MZ646074	*Promicromonospora iranensis* (MN187291)	100.0	Actinobacteria	-
06-Lb11/2	MZ646075	*Arthrobacter* sp. (KY476520)	100.0	Actinobacteria	-
07-Lb11/2	MZ646076	*Arthrobacter agilis* (JN934384)	99.9	Actinobacteria	-
08-Lb11/2	MZ646077	*Rathayibacter caricis* (LN774722)	100.0	Actinobacteria	+
10-Lb11/2	MZ646078	*Staphylococcus hominis* (MT487620)	100.0	Firmicutes	-
11-Lb11/2	MZ646079	*Rathayibacter tritici* (KR085826)	99.8	Actinobacteria	+
12-Lb11/2	MZ646080	*Kocuria palustris* (MT534060)	100.0	Actinobacteria	-
13-Lb11/2	MZ646081	*Arthrobacter agilis* (KF924209)	99.9	Actinobacteria	-
14-Lb11/2	MZ646082	*Rhodococcus cercidiphylli* (KY056167)	100.0	Actinobacteria	+
15-Lb11/2	MZ646083	*Microbacterium* sp. (MN889292)	100.0	Actinobacteria	+
16-Lb11/2	MZ646084	*Bacillus aryabhattai* (MH041178)	100.0	Firmicutes	-
17-Lb11/2	MZ646085	*Massilia aurea* (LN880088)	99.8	Betaproteobacteria	+
18-Lb11/2	MZ646086	*Bacillus* sp. (MG470665)	100.0	Firmicutes	+
19-Lb11/2	MZ646087	*Microbacterium* sp. (KM187178)	99.8	Actinobacteria	-
20-Lb11/2	MZ646088	Arthrobacter sp. (KC019196)	99.9	Actinobacteria	+
21-Lb11/2	MZ646089	*Flexivirga alba* (NR_113034)	100.0	Actinobacteria	-
22-Lb11/2	MZ646090	*Rhodopseudomonas* sp. (KF974286)	99.9	Alphaproteobacteria	-
23-Lb11/2	MZ646091	*Rhodopseudomonas palustris* (CP000463)	99.5	Alphaproteobacteria	+
24-Lb11/2	MZ646092	*Bacillus* sp. (KF582892)	100.0	Firmicutes	-
26-Lb11/2	MZ646093	*Paenibacillus* sp. (MW578439)	97.0	Firmicutes	-
27-Lb11/2	MZ646094	*Paenibacillus* sp. (KX881397)	97.0	Firmicutes	+
28-Lb11/2	MZ646095	*Paenibacillus* sp. (MW578439)	98.4	Firmicutes	+
29-Lb11/2	MZ646096	*Paenibacillus* sp. (MW578439)	95.6	Firmicutes	+
30-Lb11/2	MZ646097	*Heminiimonas* sp. (GU932947)	98.9	Betaproteobacteria	-
31-Lb11/2	MZ646098	*Kocuria palustris* (LR215141)	100.0	Actinobacteria	-
32-Lb11/2	MZ646099	*Arthrobacter agilis* (KC019195)	99.9	Actinobacteria	-
33-Lb11/2	MZ646100	*Rhodococcus cercidyphylli* (KY056167)	100.0	Actinobacteria	-
35-Lb11/2	MZ646101	*Bacillus megaterium* (MK474949)	100.0	Firmicutes	-
36-Lb11/2	MZ646102	*Tardiphaga robiniae* (MW960262)	99.4	Alphaproteobacteria	-
37-Lb11/2	MZ646103	*Bacillus subtilis* (KU904288)	99.6	Firmicutes	+
38-Lb11/2	MZ646104	*Microbacterium* sp. (KM187178)	100.0	Actinobacteria	+
40-Lb11/2	MZ646105	*Tardiphaga robiniae* (KY319041)	99.4	Alphaproteobacteria	-
42-Lb11/2	MZ646106	*Rhodopseudomonas palustris* (KT873846)	98.6	Alphaproteobacteria	-

ribosomal peptide synthetases, potentially responsible for the synthesis of siderophore-like compounds. Many *Microbacterium* strains, as shown by *in vivo* test, produce siderophores, ACC deaminase, and auxins (IAA) and can solubilize phosphate (Corretto et al., 2020). The analysis of the genome of the *Pseudarthrobacter phenanthrenivorans* strain MHSD revealed gene clusters of biosynthetic pathways for various phytohormones such as auxin, salicylic acid, ethylene, cytokinin, jasmonic acid, abscisic acid, and gibberellins (Tshishonga and Serepa-Dlamini, 2020).

Three strains of **Proteobacteria** showed an amplification product corresponding to the expected size of the PKS KS domain fragment: 03-Lb11/2 (*Methylobacterium* sp.), 23-Lb11/2 (*Rhodopseudomonas* sp.), and 17-Lb11/2 (*Massilia* sp.). Proteobacteria is a very heterogeneous group, which includes both symbionts of eukaryotes and a large number of pathogenic and opportunistic microorganisms, photo- and chemotrophic species of bacteria, autotrophs and heterotrophs. Alphaproteobacteria, as a rule, dominate proteobacteria in freshwater sponge microbiomes

(Gernert et al., 2005; Costa et al., 2013; Kaluzhnaya et al., 2011; 2012; Kaluzhnaya and Itskovich, 2014). Among Alphaproteobacteria, some phylotypes specific for communities of freshwater sponges have been identified, which indicates the possible co-evolution of sponges and some representatives of symbiotic bacteria as well as the existence of vertical transfer of symbiotic microorganisms (Taylor et al., 2007). This group of bacteria is also rather abundant in strains producing natural bioactive compounds. As an example, Alphaproteobateria of the genus *Methylobacterium* are pink pigmented, strictly aerobic, and facultative methylotrophic bacteria, commonly found in various environments; extracts obtained from these microorganisms exhibited antibacterial, cytotoxic, anticancer, and antioxidant properties (Balachandran et al., 2012; Photolo et al., 2020). Also, *Methylobacteria* have been described as beneficial bacteria owing to their function in toxic pollutant biodegradation, the stimulation of germination, and plant development (Xu et al., 2014). Another representative of Alphaproteobateria, *Rhodopseudomonas palustris*, is a photosynthetic purple non-sulfur bacteria (Austin et al., 2015) exhibiting diverse biological activities. Su et al. (2015) found an antiviral protein showing significant inhibitory activity against tobacco mosaic virus (TMV) *in vivo* and *in vitro* in the JSC-3b bacterial strain. Nookongbuta et al. (2020) demonstrated that exopolymeric substances (EPS), lipopeptides and photo-pigments extracted from the KTSSR54 strain showed antifungal activity against three rice fungal pathogens. Other representatives of this species demonstrated the ability to degrade aromatic compounds and utilize short-chain organic acids during photoheterotrophic cultivation in an anaerobic environment (Austin et al., 2015).

Betaproteobacteria are known for their morphological diversity; they are often the dominant group in lake ecosystems, but they are not numerous in sponge communities. This bacterial class includes several groups of aerobic or facultative bacteria with various metabolic capabilities (chemolithotrophs and phototrophs) (Newton et al., 2011). Representatives of the Betaproteobacteria, strains of the genus *Massilia*, also showed the ability to produce bioactive substances such as dimethyl disulfide (DMDS) that have the potential to control plant foliar diseases (Feng et al., 2016), degrade chloroacetamide herbicide (Lee et al., 2017), and inhibit pathogenic strains of *Escherichia coli* and *Pseudomonas aeruginosa* (Dahal et al., 2021).

In five of the nine isolated **Firmicutes** strains, there was a positive PCR signal for the presence of genes of biologically active metabolites: 18-Lb11/2, 37-Lb11/2 (*Bacillus* sp.), 27-Lb11/2, 28-Lb11/2, and 29-Lb11/2 (*Paenibacillus* sp.).

Firmicutes as a part of lake communities are usually found among the minor phyla and mainly inhabit the bottom layer and sediments. However, some strains of this phylum (for example, species of the genera *Pseudomonas* and *Bacillus*) are often found among cultured bacteria in aquatic and sponge communities (Newton et al., 2011). Representatives of Firmicutes, along with Actinobacteria, are known as producers of biologically active metabolites (Su, 2014).

The presence of genes for the synthesis of biologically active substances in the strains of the genus *Bacillus* is expectable because many representatives of this genus are known as producers of various bioactive metabolites, including antibiotics and toxins such as bacilysin and fengymycin, iturin and iturin-like substances, surfactin, and bacillomycin (Fickers, 2012; Zeng et al., 2016). For example, the *Bacillus axarquiensis* strain TUBP1 exhibited antifungal activity against *Verticillium dahliae*, a fungus that causes a soil-borne disease of cotton crops (Zeng, et al., 2016). Various strains of *Bacillus tequilensis* exhibited antibacterial activity due to their ability to synthesize alkaline protease (Khan et al., 2019) as well as lipoproteins and biosurfactants (Akinsanya et al., 2019).

Determination of the amplified gene sequences, as well as the identification of the antibiotic activity of the selected strains (in relation to test cultures of opportunistic microorganisms), is the next stage of this study.

5. Conclusions

In this study, we identified 35 bacterial strains isolated from the symbiotic community of the freshwater sponge, *L. baicalensis*. Representatives of 16 genera belonging to three bacterial phyla were detected, which indicates the presence of a significant diversity of cultured microorganisms in the *L. baicalensis* community. Their PCR screening was carried out to select cultures potentially capable of producing secondary metabolites of polyketide nature. PCR product corresponding in size to a fragment of the PKS gene was found in 15 strains. Therefore, the proposed method is convenient for the preliminary analysis of a large number of systematically heterogeneous strains. In future research, the biological activity of strains showing a positive PCR signal can be investigated using microbiological, biochemical, and analytical methods. This work contributes to the study of the diversity and biotechnological potential of the cultured microorganisms of the Baikal sponges.

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Conflicts of interest

The authors declare no conflicts of interest.

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